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Photostabilized titanium dioxide and a fluorescent brightener as adjuvants for a nucleopolyhedrovirus *

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Abstract. Titanium dioxide (TiO_2) reflects ultraviolet light, and so could be expected to protect the occlusion bodies (OBs) of nucleopolyhedroviruses (NPVs) from degradation by sunlight. However, in the presence of sunlight and water, TiO_2 catalyzes the formation of hydrogen peroxide, which can degrade OBs. We tested microfine TiO_2 that had been photostabilized (particles were coated to prevent catalytic activity), as a UV protectant for the OBs of the NPV of *Helicoverpa zea* (Boddie). In the absence of UV, activity of the OBs was reduced by nonphotostabilized TiO_2 but was unaffected by photostabilized TiO_2 or by zinc oxide (ZnO). None of these materials influenced larval feeding rates. Under simulated sunlight, photostabilized TiO_2 protected the OBs to a greater degree than did ZnO. Photostabilized TiO_2 was compatible with a viral enhancer, the fluorescent brightener Blankophor HRS. Under simulated sunlight, both materials increased activity of the OBs, relative to OBs with neither material, in a largely additive manner. In bioassays of foliage collected from field plots of lima bean plants sprayed with OBs with or without one or both of these materials, TiO_2 increased persistence of the OBs, but Blankophor HRS had no significant effect.

Key words: fluorescent brightener, *Helicoverpa zea*, nucleopolyhedrovirus, photostabilized, titanium dioxide, ultraviolet

Nucleopolyhedroviruses (NPVs) have been the subject of considerable research over the last few decades as microbial control agents for insect pests, particularly caterpillars (Lepidoptera) and sawflies (Hymenoptera) (Entwistle and Evans, 1985; Maramorosch and Sherman, 1985; Granados and Federici, 1986; Adams and McClintock, 1991; Hunter-Fujita et al., 1998). However, to date, the use of NPVs as microbial control agents has been limited by a number of factors, particularly the susceptibility of their occlusion bodies (OBs) to degradation by the ultraviolet (UV) portion of sunlight (Jaques,

Note: Use of trade names does not imply endorsements of products named nor criticism of similar ones not mentioned.

1977, 1985; Shapiro, 1995). UV degrades most, if not all, microbial control agents, but viruses are particularly sensitive (Ignoffo et al., 1977). Thus, extensive research has been done on materials that can protect NPVs and other microbial control agents from UV in the field, before ingestion by the target insect (reviewed by Jaques, 1977, 1985; Shapiro, 1995; Burges and Jones, 1998; Hunter-Fujita et al., 1998).

Burges and Jones (1998) classified UV protectants as absorbers, reflectors, and antioxidants. Absorbers and reflectors are intended to prevent UV from reaching the microbial agent, while antioxidants counteract oxidative chemicals created by the interaction of UV with water and/or the substrate that might otherwise damage the microbial agent. However, little has been published on the use of reflectors with OBs of NPVs. Among those materials that have been tested as reflectors are aluminum powder (Ignoffo and Batzer, 1971) and titanium dioxide (TiO_2) (Bull et al., 1976), with the latter appearing the more effective. TiO_2, however, has been studied extensively as a sunscreen for use on human skin (Anderson et al., 1997; Fairhurst and Mitchnick, 1997).

While TiO_2 reflects UV, it also has a property that would be undesirable in a formulation of an NPV: it is a photocatalyst. In the presence of water and light, TiO_2 catalyzes the formation of hydrogen peroxide (H_2O_2) (Hoffman et al., 1995). Reactive oxygen species, such as peroxides, are known to inactivate OBs of NPVs (Ignoffo and Garcia, 1994; Hunter-Fujita et al., 1998), though the role of these chemicals in inactivation of OBs on plant surfaces in the field has not been determined. Thus, TiO_2 has the potential to reduce the activity of OBs even though it can reflect UV. The catalytic activity of TiO_2 can be eliminated, however, by "photostabilizing," or coating of the TiO_2 particles with materials such as silica or stearate (Fairhurst and Mitchnick, 1997). Herein, we report the results of tests of photostabilized TiO_2 as a UV protectant for OBs of the NPV of the corn earworm, *Helicoverpa zea* (Boddie) (HzSNPV).

Diaminostilbene disulfonic acid-based fluorescent brighteners, or optical brighteners, which absorb energy in the form of UV and re-emit it as visible light, function as UV absorbers for OBs (Shapiro, 1992). In addition, certain of these materials are of particular interest because, independently of their UV protectant activity, they are also strong enhancers of the activity of many NPVs (Shapiro, 1995; Farrar and Ridgway, 1997; Hamm, 1999). For example, in laboratory bioassays, median lethal concentrations (LC_{50}s) for the OBs of the NPV of the gypsy moth, *Lymantria dispar* (L.) (LdMNPV), were reduced by as much as 1,837 – fold with the addition of a fluorescent brightener (Shapiro and Robertson, 1992; Shapiro et al., 1992). Given the potential benefit of the use of these materials, through a different mechanism

than of TiO_2, we also tested a fluorescent brightener in combination with photostabilized TiO_2.

Materials and methods

Insects, plants, TiO_2 and virus. We obtained all insects from a stock culture (Insect Biology and Population Management Research Laboratory, USDA-ARS, Tifton, GA). Larvae were reared, before and after treatment, on artificial diet (King and Hartley, 1985).

We used lima bean, *Phaseolus lunatus* L., cv. 'Maffei 15,' as the host plant. For laboratory bioassays, plants were grown in a greenhouse in 10-cm diam pots, 2 to 4 plants per pot, with a commercial potting medium (Pro Mix BX®, Premier Brands, Red Hill, PA). Plants were grown under a regime of 24 ± 3 °C, with the photoperiod supplemented to 16:8 (L:D) h by low-pressure sodium vapor lamps, and were fertilized weekly (Peters Professional 20-20-20®, Grace-Sierra, Milpitas, CA). Plants were 5 to 6-wk old when used.

Photostabilized TiO_2 (Nano Tek® titanium dioxide) was obtained from Nanophase Technologies (Burr Ridge, IL). This material is water dispersible, and was obtained as a mixture of water and coated TiO_2 particles (50% water by weight). Nonphotostabilized TiO_2 and zinc oxide (ZnO) (both dry powders) were also obtained from Nanophase Technologies. Photostabilized TiO_2 (Cardre 72248J) was also obtained from Cardre (South Plainfield, NJ). This material was obtained dry, and is not dispersible in water without added wetting agents. Except as noted below, Nanophase Technologies materials were used in all tests.

We obtained samples of HzSNPV (Gemstar®), labeled to contain 2.00×10^9 OBs/ml, from Thermo Trilogy (now Certis USA, Columbia, MD).

UV source. We used a sunlight simulator, Suntester CPS+® (Atlas Electric Devices, Chicago, IL), as a UV source. This device uses a xenon lamp with a filter to illuminate a chamber of 20 cm × 28 cm in area with light similar to the solar spectrum at the Earth's surface, from UV-B through the visible portion of the spectrum. McGuire et al. (2000) tested this device and found that the rate of degradation of an NPV under simulated sunlight was similar to that under natural sunlight when exposure was expressed as cumulative total energy (joules/m^2). Irradiance was set at 500 W/m^2. A UV meter (UVX®, Ultraviolet Products, San Gabriel, CA), set to measure UVA (365 nm), placed on the bottom of the chamber gave a reading of 2750 μW/cm^2 at this setting. For comparison, a reading of 2820 μW/$cm^2$was obtained from sunlight at noon in June at Beltsville, MD. The chamber

containing the lamp is cooled by a current of chilled air, as well as by chilled water circulating under a pan at the bottom of the chamber. To prevent treated leaf disks (see below) form being moved and/or desiccated by the air current, disks were held in a box with a top made of UV-transparent acrylic plastic, 2.5 mm thick (UVT®, Polycast Technology, Stamford, CT), during exposure to UV. This plastic transmits >95% of UV energy (Ridgway and Farrar, 1999). The box is 2.5 cm deep with a thin aluminum flashing bottom, and was made to fit inside the pan at the bottom of the UV-exposure chamber. Moist absorbent paper under, as well as inside, the box allowed excess heat to be conducted away from the chamber by the circulating chilled water.

Adjuvant treatments. Treatments included in laboratory tests are listed in Table 1. All materials were mixed with distilled water. All concentrations are percentages, by dry weight per unit weight of water. All treatments also included 0.01% Triton X-155® (wetting agent; Union Carbide, Danbury, CT).

Experiment 1. We measured feeding rates of *H. zea* larvae on leaf disks treated with TiO_2 in the absence of OBs of HzSNPV by the method of Farrar and Ridgway (1994). Late first instars were held on moist filter paper in cells of plastic bioassay trays (Bio-BA 128®, CD International, Pitman, NJ) and starved for 18 h. Only those larvae that molted to the second instar during this time interval were used. Leaf disks of 9 mm diam were used.

Ten larvae were tested per treatment, and the test was replicated three times, for a total of 30 larvae per treatment. Relative consumption rate (RCR) was then calculated as dry weight of food eaten divided by initial dry weight of each larva (Farrar et al., 1989). Data were analyzed by analysis of variance (ANOVA) through the PROC GLM procedure of SAS (SAS Institute, 1988). Each larva was treated as an observation. Independent variables included treatment and replication (treated as a block variable), while RCR was the dependent variable. In this and all other experiments, means and standard errors were calculated from untransformed data through the PROC MEANS procedure (SAS Institute, 1988) and results are presented as such.

Experiment 2. To test for adverse effects of non-photostabilized TiO_2 on HzSNPV, we compared photostabilized and non-photostabilized TiO_2, and ZnO, in the absence of UV. In bioassays of the virus, 28 OBs (LD_{90}, Farrar and Ridgway, 1999) in 2 μl water were pipetted onto individual leaf disks (6 mm diam), which individual larvae were allowed to consume completely, thus providing a known dose. Only larvae that consumed the entire disk within 48 h were then transferred to artificial diet and scored for mortality after 7 d (Farrar and Ridgway, 1999).

Table 1. Treatments tested in the laboratory as adjuvants for OBs of HzSNPV

Experiment number	Treatment	Concentration(s), of adjuvants, %[a]
1	Photostabilized TiO_2	1.00
	Nonphotostabilized TiO_2	1.00
	ZnO	1.00
	Control, no adjuvant	–
2	Photostabilized TiO_2	0.50, 1.00, 2.00
	Nonphotostabilized TiO_2	0.50, 1.00, 2.00
	ZnO	0.50, 1.00, 2.00
	Controls, each of the above, 0 OBs	1.00
	OBs only	–
3	Photostabilized TiO_2	0.10, 0.25, 0.50, 1.00
	ZnO	0.10, 0.25, 0.50, 1.00
	Controls, each of the above, 0 OBs	1.00
	OBs only	–
4, test 1	Photostabilized TiO_2	0.00, 0.25, 0.50, 1.00
	Blankophor HRS	0.00, 0.10, 0.25, 0.50
4, test 2	Photostabilized TiO_2	0.00, 0.05, 0.10, 0.25
	Blankophor HRS	0.00, 0.05, 0.10, 0.25
	Controls, each of the above, 0 OBs	Highest concentrations
	OBs only	–
5	NanoTek photostabilized TiO_2	0.50
	Cardre photostabilized TiO_2	0.50
	Controls, each of the above, 0 OBs	0.50
	OBs only	–

[a](Dry weight of adjuvant/unit weight water) × 100.

Twenty-four larvae (late first instars to very early second instars) were placed on foliage of each treatment. Larvae were held in an incubator at 27 °C under illumination by fluorescent lighting with a photoperiod of 16:8 (L:D) for 48 h. The test was replicated four times.

Mortality caused by 28 OBs per larva only was treated as original activity (OA). Original activity remaining (OAR) was calculated by dividing mortality on other treatments by OA. OAR was normalized by arcsine $\sqrt{\%}$ transformation and analyzed by ANOVA (PROC GLM) with replication, adjuvant treatment, concentration, and the interaction of treatment and concentration as independent variables. Because a significant interaction was

found (see results), adjuvant treatments were also analyzed separately for effects of concentration.

Experiment 3. We compared ZnO and photostabilized TiO_2 as UV protectants for HzSNPV. In other tests (R.R.F., unpublished data), exposure of disks treated with 28 OBs per disk to UV in the sunlight simulator at 500 W/m^2 for 26.5 min. reduced activity by 90%. In this test, this dosage and an exposure interval of 3 h were used to test TiO_2 and ZnO. (If lima bean leaf disks are held in the sunlight simulator for >3 h, they begin to desiccate, despite being kept on cool, moist paper towels.)

Treatments included 28 OBs with no adjuvants, both exposed to UV and unexposed, as well as adjuvant treatments. This test was replicated four times. OAR was calculated relative to mortality caused by 28 OBs, unexposed, and normalized as described above. Data were analyzed by factorial ANOVA with replication, adjuvant type, concentration, and the interaction of adjuvant and concentration as independent variables.

Experiment 4. The interaction of photostabilized TiO_2 with a fluorescent brightener was tested. Blankophor HRS® (Bayer, Rock Hill, SC) was the selected fluorescent brightener because previous tests indicated that it was an effective enhancer of HzSNPV in the laboratory and not a feeding deterrent to *H. zea* (R.R.F., unpublished data). Treatments (Table 1) were included in all combinations, all exposed to UV. Sixteen larvae per treatment combination were offered disks. This test was otherwise similar to Experiment 2. It was replicated four times.

Because mortality was $>90\%$ on many of the treatments in the above test (see Results), a second test was conducted with lower concentrations of both materials. This test was replicated six times.

Data from each test were analyzed by factorial ANOVA with replication, concentration of TiO_2, concentration of Blankophor HRS, and the interaction of the concentrations of these two materials as factors and percent mortality (normalized by arcsine $\sqrt{\%}$ transformation) as the dependent variable. OAR was not used because Blankophor HRS can enhance the activity of HzSNPV; mortality caused by treatments with this material can thus be greater than that caused by 28 OBs alone, resulting in OAR $>100\%$, for which an arcsine value cannot be calculated. A significant interaction between concentrations of TiO_2 and Blankophor HRS was found in both tests (see Results). Because this interaction appeared to be the result of large differences between the treatment with 28 OBs only, exposed to simulated sunlight, and the other exposed treatments, data were also analyzed with this treatment omitted.

Experiment 5. In 2000, Nano Tek TiO_2 became unavailable. We therefore obtained Cardre 72248J TiO_2 as a substitute. As these materials differ, particularly in dispersibility in water, a preliminary test was conducted to compare them prior to proceeding with further tests. To disperse Cardre TiO_2 in water, a concentrate was prepared by adding 5g TiO_2 and 1ml Triton X-155 to 94 ml distilled water and mixing in a small blender. This concentrate was then diluted further as required. The two materials were compared as in Experiment 3. This test was replicated five times.

Experiment 6. Lima beans, cv. 'Maffei 15,' were planted on the campus of the University of Maryland Eastern Shore in Princess Anne, MD on 28 July 1999 and 24 July 2000. Seeds were planted on 76 cm rows. The field was divided into plots measuring 8 m long by two rows wide in 1999 and 4.5 m by two rows wide in 2000. There was one border row between plots across rows and 2 m between plots within rows. The experimental design was a randomized complete block with four blocks.

A CO_2-pressurized backpack sprayer (KQ-25, Weed Systems, Inc., Keystone Heights, FL) was used to apply treatments. The sprayer had three Tee Jet 8002® flat fan nozzles (Spraying Systems, Dillsburg, PA), one ca. 30 cm above the row, and two dropped nozzles 60 cm apart, aimed horizontally at the row, with the spray fan vertical. It was calibrated to deliver 187 liters/ha (20 gal/acre) at a pressure of 2.11 kg/cm^2 (30 lb/in^2) and a walking speed of 6.4 km/h (4 mi/h).

In 1999, treatments included control, and HzSNPV at 2.47×10^{11} OB/ha and 2.47×10^{12} OB/ha (10^{11} and 10^{12} OB/acre, respectively). Also included were treatments with 2.47×10^{11} OB/ha with 0.10% or 0.25% Blankophor HRS, 1.00% Nano Tek TiO_2, or 0.25% Blankophor HRS plus 1.00% TiO_2. A spreader, Kinetic® (Setre Chemical, Memphis, TN) was included in all treatments at 0.125%.

In 2000, Cardre TiO_2, prepared as a concentrate as described above, was substituted for Nano Tek material. Treatments included OBs only at the same rates as in 1999, 2.47×10^{11} OB/ha with Blankophor HRS at 0.25% or 0.50%, TiO_2 at 0.50%, or TiO_2 plus Blankophor HRS at 0.25% and 0.50%, respectively. Joint Venture® (Helena Chemical, Memphis, TN), a spreader/sticker marketed for use with biological insecticides, was included in all treatments at 0.125%.

Within 5 min. following application of the virus, foliage was collected from each plot for bioassay. Four fully expanded leaflets were collected from the uppermost parts of different plants in each plot and returned to the laboratory. Four pieces of about 1 cm^2 each were cut from each leaflet (16 pieces per plot). Pieces were placed individually in cells of plastic bioassay trays with

moist filter paper and one late first instar to very early second instar. Larvae were held at 24 ± 3 °C for 48 h. All larvae were then transferred to artificial diet. (The pieces of foliage were too large to be consumed entirely.) Larvae were held at 24 ± 3 °C for eight additional days and scored for mortality. This bioassay was repeated at 24, 48, 72, and 96 h after application of virus.

Percentage mortality was calculated, adjusted for control mortality with Abbott's (1925) formula, and normalized by arcsine $\sqrt{\%}$ transformation. Data were analyzed by factorial ANOVA with block, days after application, and rates of TiO_2 and Blankophor HRS as independent variables (PROC GLM, SAS Institute, 1988). Data were also analyzed by ANOVA with treatments as class variables and means separated by the least significant difference (LSD) test (PROC GLM, SAS Institute, 1988).

Results

Experiment 1. Consumption of lima bean foliage by *H. zea* larvae was unaffected by any treatment ($F = 0.30$; df = 3, 110; $P = 0.8260$). RCR (±SE) for control (untreated), nonphotostabilized TiO_2, photostabilized TiO_2, and ZnO was 9.46 (±0.923), 9.13 (±0.735), 8.49 (±0.837), and 9.09 (±0.792), respectively.

Experiment 2. In the absence of UV, the activity of HzSNPV OBs was reduced by the addition of nonphotostabilized TiO_2, but was not significantly affected by photostabilized TiO_2, or by ZnO (Figure 1). Over all treatments, activity was unaffected by adjuvant ($F = 0.36$; df = 2, 27; $P = 0.7031$), but was affected by concentration of adjuvants ($F = 8.90$; df = 1, 27; $P = 0.0060$) and by the interaction thereof ($F = 7.85$; df = 2, 27; $P = 0.0021$). Activity of OBs declined with increasing rates of nonphotostabilized TiO_2 ($F = 11.95$; df = 1, 11; $P = 0.0106$), but was unaffected by increasing rates of photostabilized TiO_2 ($F = 0.33$; df = 1, 11; $P = 0.5840$) or of ZnO ($F = 0.04$; df = 1, 11; $P = 0.8463$). Mortality caused by 28 OB only (OA) was 96.0% (±2.77%). No mortality occurred on the control treatment.

Experiment 3. Under simulated sunlight, photostabilized TiO_2 protected the OBs to a greater degree than did ZnO (Figure 2). When all treatments were analyzed together, OAR was affected by adjuvant ($F = 30.01$, df = 1, 31; $P = 0.0001$), concentration of adjuvant ($F = 38.06$, df = 1, 31; $P = 0.0001$), and by the interaction thereof ($F = 5.29$, df = 1, 31; $P = 0.0301$). Separately, the effect of concentration was significant for both materials, but was stronger for ZnO ($F = 55.02$, df = 1, 15; $P = 0.0001$) than for TiO_2 ($F = 5.21$, df = 1, 15; $P = 0.0433$). Mortality caused by 28 OBs not exposed to simulated

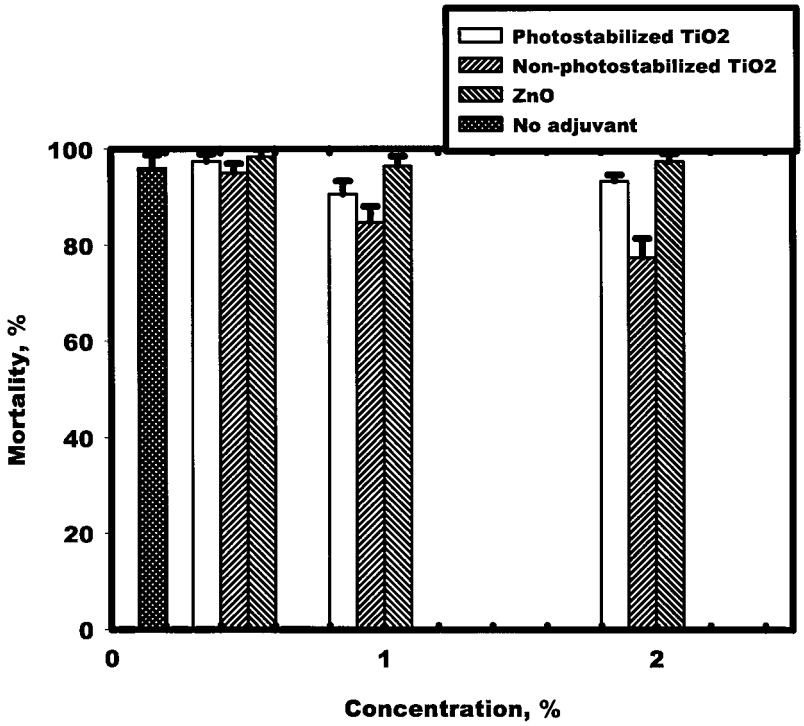

Figure 1. Mean (± SE) mortality of corn earworm larvae fed OBs of HzSNPV with and without varying concentrations of photostabilized or nonphotostabilized TiO_2 or ZnO in the absence of UV.

sunlight (OA) was 89.3% (±3.45%). No mortality was caused 28 OBs only, exposed to simulated sunlight, or by the control.

Experiment 4. In the first test of combinations of photostabilized TiO_2 and Blankophor HRS under simulated sunlight, increasing concentrations of both materials increased mortality (Figure 3A, Table 2). However, when the treatment with 28 OBs only, exposed to simulated sunlight (no mortality), was excluded from the analysis, only the rate of Blankophor HRS affected mortality. The interaction of the concentrations of the two materials was significant only when the 28 OBs only treatment was included. Mortality caused by 28 OBs only, not exposed to simulated sunlight (OA), was 91.7% (±1.67%). Control mortality was 1.56%.

In the second test, in which concentrations of both materials were lower, mortality was affected by the concentration of both materials, both when the treatment with 28 OBs only was included, and when it was excluded (Figure 3B, Table 1). The interaction was again significant only when the

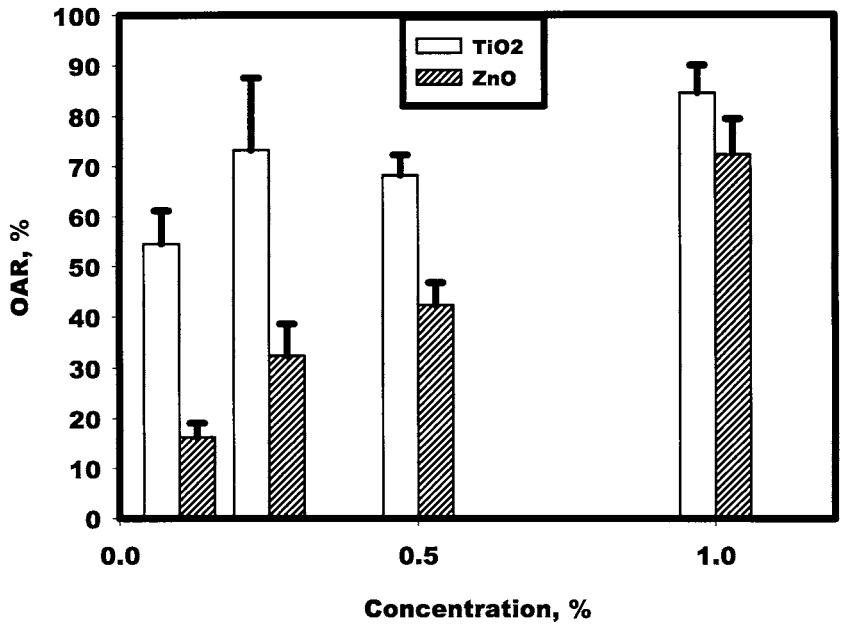

Figure 2. Mean (± SE) original activity remaining (OAR) of OBs of HzSNPV exposed to UV for 3 h with varying concentrations of photostabilized TiO_2 or ZnO. Original activity of OBs alone with no UV was 89.3% (SE = 3.45%). Activity of OBs under UV with no adjuvants was 0.0%.

Table 2. Statistical results from tests of the effects of combinations of photostabilized TiO_2 and Blankophor HRS on the activity of OBs of HzSNPV under simulated sunlight. Data were analyzed both with and without the treatment with OBs but with neither TiO_2 nor Blankophor HRS. (Means are presented in Figure 3.)

Test	Fig.[a]	Independent variable	Results with OBs-only treatment			Results without OBs-only treatment		
			F	df	*P*	*F*	df	*P*
1	A	Rate of TiO_2	9.75	1, 57	0.0028	0.05	1, 53	0.8175
		Rate of Blankophor HRS	45.25	1, 57	0.0001	21.53	1, 53	0.0001
		Interaction	5.25	1, 57	0.0257	0.02	1, 53	0.9018
2	B	Rate of TiO_2	42.37	1, 86	0.0001	18.23	1, 80	0.0001
		Rate of Blankophor HRS	90.74	1, 86	0.0001	60.69	1, 80	0.0001
		Interaction	5.97	1, 86	0.0166	0.26	1, 80	0.6097

[a]Part of Figure 3 showing corresponding means.

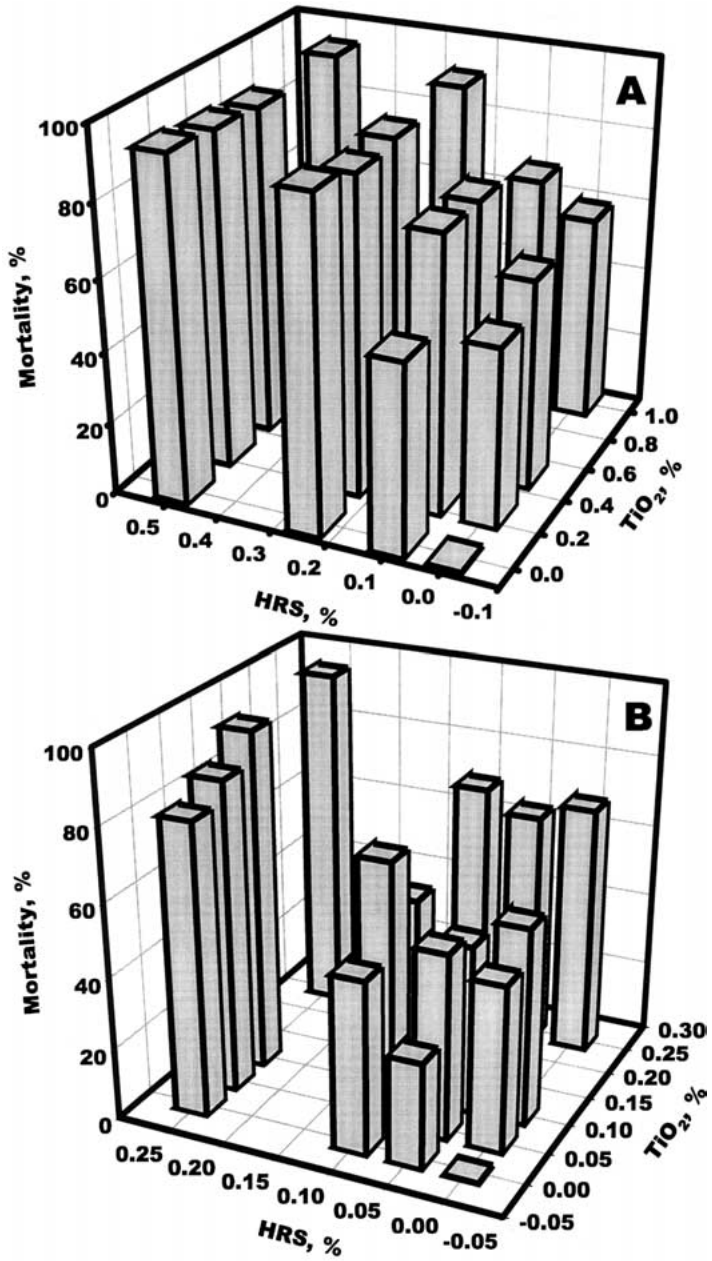

Figure 3. Mortality of corn earworm larvae fed OBs of HzSNPV exposed to UV for 3 h with and without varying combinations of both photostabilized TiO_2 and Blankophor HRS at higher (A) or lower (B) concentrations. Original activity of OBs alone with no UV was 91.7% (SE = 1.67%) in the former test; 88.4% (SE = 6.50%), in the latter test.

28 OBs only treatment was included. Mortality caused by 28 OBs only, not exposed to simulated sunlight (OA), was 88.4% (±6.50%). No control mortality occurred.

Experiment 5. OAR for virus treatments exposed to simulated sunlight with Nano Tek and Cardre TiO_2 was 42.8% (± 6.87%) and 52.8% (± 5.36%), respectively, with no significant difference ($F = 1.00$; df = 1, 4; $P = 0.3732$). Original activity of unexposed 28 OBs only was 79.4% (±2.10%). No mortality occurred on the treatment with 28 OBs only, exposed to simulated sunlight, or in the controls.

Experiment 6. Activity of OBs on lima bean plants in the field declined on all treatments over the course of the tests. However, persistence was greater on those treatments that included TiO_2. The effect of concentration of TiO_2 on mortality on treatments with 2.47×10^{11} OB/ha was significant in 1999 ($F = 9.05$; df = 1, 81; $P = 0.0035$) (Figure 4) as well as in 2000 ($F = 26.48$; df = 1, 115; $P = 0.0001$) (Figure 5). Mortality was unaffected by concentration of Blankophor HRS ($F = 0.04$; df = 1, 81; $P = 0.8356$ in 1999; $F = 3.30$; df = 1, 115; $P = 0.0718$ in 2000) or by the interaction of the concentrations of Blankophor HRS and TiO_2 ($F = 0.11$; df = 1, 81; $P = 0.7430$ in 1999; $F = 0.52$; df = 1, 115; $P = 0.4702$ in 2000). In 1999, mortality of larvae on the control was 1.9, 3.7, 4.4, 0.0 and 0.0% on foliage collected 0, 24, 48, 72, and 96 h, respectively, after treatment. In 2000, control mortality was 1.7% on foliage collected 0 h after treatment and 0.0% on foliage collected at other times.

When data were analyzed with treatment as a class variable, mortality was affected by treatment in both years ($F = 7.79$; df = 1, 96; $P = 0.0001$ in 1999; $F = 17.29$; df = 1, 132; $P = 0.0001$ in 2000). Treatments with 2.47×10^{11} OB/ha plus TiO_2 were not significantly different ($P > 0.05$) from the treatments with 2.47×10^{12} OB/ha in either year. Treatments with the 2.47×10^{11} OB/ha and Blankophor HRS did not differ ($P > 0.05$) from those with 2.47×10^{11} OB/ha alone in either year. The treatments with 2.47×10^{11} OB/ha only and those with 2.47×10^{12} OB/ha were significantly different ($P < 0.05$) in both years.

Discussion

Our results indicate that TiO_2 can protect OBs of HzSNPV from UV, and, further, that photostabilization improves compatibility of TiO_2 with the OBs. We saw no indication of effects on feeding that might result in incompatibility of TiO_2 with NPVs.

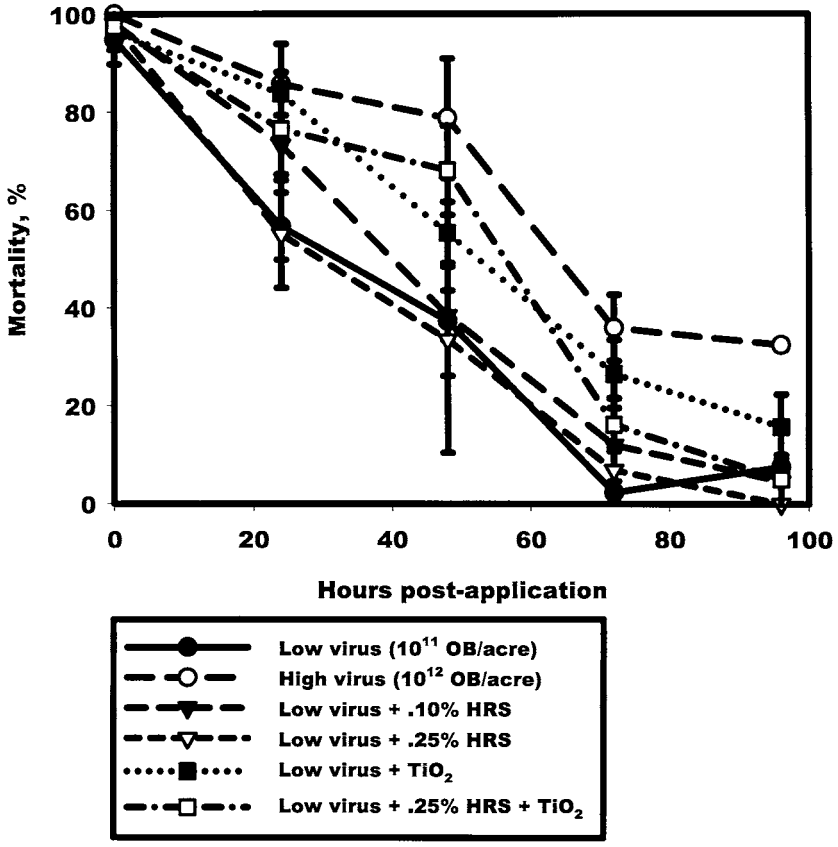

Figure 4. Mortality of corn earworm larvae on foliage of lima bean collected from the field 0 to 4 d after treatment with OBs of HzSNPV with or without TiO_2 and Blankophor HRS, University of Maryland Eastern Shore, Princess Anne, MD, 1999.

Nonphotostabilized TiO_2 is known to catalyze the production of H_2O_2, (Hoffman et al., 1995), which, in turn, is known to degrade OBs of NPVs (Ignoffo and Garcia, 1994; Hunter-Fujita et al., 1998). The decline in activity of OBs with increasing concentrations of nonphotostabilized TiO_2 in the absence of UV, compared with no effect of photostabilized TiO_2 (Figure 1), is consistent with degradation of the virus by catalytically produced H_2O_2. The lack of an effect of ZnO, which does not catalyze the production of H_2O_2, is also consistent with this effect. Our evidence of this effect is, though, indirect because hydrogen peroxide was not measured. Nevertheless, we believe that photostabilized TiO_2 should be more compatible with at least the OBs of this NPV than would be nonphotostabilized TiO_2.

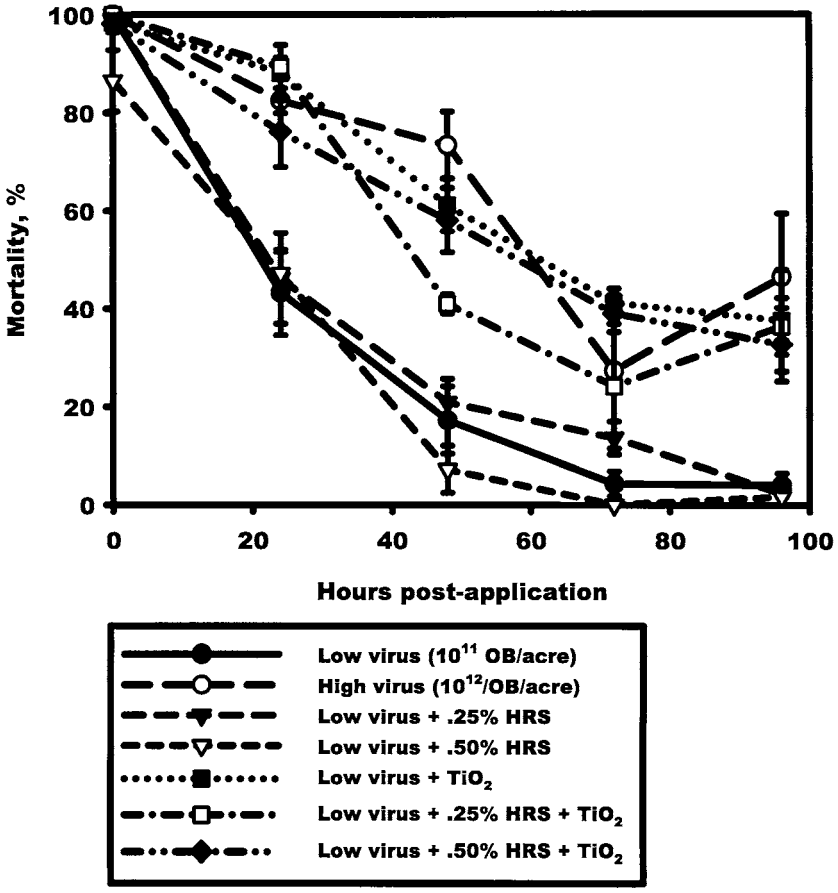

Figure 5. Mortality of corn earworm larvae on foliage of lima bean collected from the field 0 to 4 d after treatment with OBs of HzSNPV with or without TiO_2 and Blankophor HRS, University of Maryland Eastern Shore, Princess Anne, MD, 2000.

In our laboratory tests, TiO_2 provided greater protection of OBs than did ZnO, especially at lower concentrations (Figure 2). ZnO also was harder to suspend in water; more agitation of this suspension during application to leaf disks was necessary than was necessary with TiO_2. ZnO was therefore not tested further.

TiO_2 appears to be compatible with the fluorescent brightener, Blankophor HRS. In laboratory bioassays (Figure 3, Table 1), the interaction between rates of these two materials was significant only when the treatment of OBs with neither material, exposed to simulated sunlight, was included. The difference between this treatment, which had no protection from UV, and

the other treatments was relatively large. Among the other treatments, the combined effects appear to be additive.

The effect of TiO_2 as a UV protectant in the laboratory was consistent over a range of concentrations. A strong effect of concentration of TiO_2 was evident only in the second test of combinations of TiO_2 and Blankophor HRS (Figure 3B, Table 1), in which very low concentrations were included. A decline in efficacy was seen primarily at concentrations of $<0.25\%$; some effect still occurred at 0.05%. These results indicate that relatively small amounts of TiO_2 may be needed to protect NPVs from sunlight. In the laboratory, at least some activity was preserved for 3 h, whereas in the absence of protectants activity of OBs under our UV exposure regime is lost in about 30 min (R.R.F., unpublished data).

Our results indicate that photostabilized TiO_2 from Nanophase Technologies and that from Cardre were comparable in effectiveness as UV protectants for HzSNPV.

Results of the field persistence tests are consistent with results of the laboratory tests with regard to TiO_2. In both years, levels of activity of 2.47×10^{11} OB/ha with TiO_2 were comparable to those of 2.47×10^{12} OB/ha with no TiO_2 or brightener.

No significant effect of Blankophor HRS was seen in the field persistence test in either year, despite significant effects seen in the laboratory. Other field studies of brighteners on field and vegetable crops have also been inconsistent. In bioassays of field-collected foliage treated with another brightener, Blankophor BBH®, against the beet armyworm, *Spodoptera exigua* (Hübner) on collard, *Brassica oleracea* L., significant effects of the brightener on viral activity were seen in some tests but not in others (Farrar et al., 1999). Vail et al. (1993) tested several NPVs with and without a brightener and a feeding stimulant against the tobacco budworm, *Heliothis virescens* (F.), and *H. zea* on cotton, *Gossypium hirsutum* L., in three localities. They obtained increased activity with both the enhancer and feeding stimulant in some tests but found no differences in other tests. Vail et al. (1999), using bioassays of cotton foliage collected over several days, found little effect of a brightener on persistence of an NPV. However, Hamm et al. (1994) obtained higher levels of mortality of larvae of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), by the addition of a brightener to sprays of the fall armyworm NPV applied to whorl-stage corn, *Zea mays* (L.), in the field.

The titanium dioxide itself is not expected to decompose under field conditions, though persistence of this material was not measured. Titanium dioxide is used extensively as a pigment in paints, which are exposed to the environment for long periods of time.

Hunter-Fujita et al. (1998) list TiO_2 as a material that has been used as a UV reflector with NPVs, but then state that the use of this material "may be impermissible as titanium is now considered an environmental pollutant." However, they give no references on environmental hazards of TiO_2. The Hazardous Substances Data Bank (The National Library of Medicine, 1999), which summarizes investigations of hazards of chemicals, lists no major risks associated with TiO_2 other than as a nuisance dust. Similarly, elemental titanium has few risks other than combustibility under certain conditions. The addition of small amounts of TiO_2 to aqueous or other liquid carriers of NPVs would not be likely to produce significant amounts of dust. TiO_2 has also been studied extensively for use in sunscreens for human skin, with no major problems (Anderson et al., 1997; Fairhurst and Mitchnick, 1997). Photostabilized TiO_2 would thus appear to pose little, if any, environmental risk.

Photostabilized TiO_2 could be useful as a UV protectant for microbial pest control agents other than NPVs, such as bacteria and fungi. We have only tested it with OBs of HzSNPV; however, as a reflector of UV, there is no reason to suspect that it would not be useful in other systems where UV is a concern.

In at least some systems, the materials that we tested may at present be prohibitively expensive. The wholesale price of Cardre 72248J TiO_2, as of August 2001, was \$40.13/kg (\$18.06/lb). At 0.25% of 187 liters/ha (20 gal/acre), the cost of this material would be \$18.76/ha (\$7.59/acre). However, given technical feasibility of using photostabilized TiO_2 with NPVs, it may be possible to develop lower cost materials specifically for agricultural use.

In summary, photostabilized TiO_2 holds promise as a UV protectant for NPVs. It does not interfere with feeding and is more compatible with NPVs than is nonphotostabilized TiO_2. It is compatible with fluorescent brighteners and is an effective UV protectant at low concentrations. Moreover, it is apparently safe to use. Further testing against populations of insects in the field is planned.

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